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Commentary

Exciting Developments in the Immunology of Fungal Infections

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The last decade has brought significant advances in our understanding of antifungal immunity, which offer hope for the development of novel immunotherapeutics. In this commentary, we provide a snapshot of the protective innate and adaptive components of antifungal immunity and highlight several recent topics of interest, placing in context the three associated reviews in this issue of *Cell Host & Microbe*.

Introduction

Of all the pathogens of man, fungi are the least well studied and understood. The reasons for this are largely historical; healthy people rarely get life-threatening fungal infections, and although many suffer from superficial fungal infections of the skin and mucosa, these infections are often treatable with current antifungal agents. However, alterations in immune status or breaching of physical barriers can render individuals susceptible to life-threatening invasive fungal diseases, and the incidence of these types of infections has increased substantially in the last few decades due to the HIV/AIDS pandemic and modern immunosuppressive and invasive medical interventions. Moreover, invasive fungal diseases are difficult to diagnose and treat and frequently have mortality rates exceeding 30% to 50%, despite the introduction of novel classes of antifungals (azoles and echinocandins) in clinical practice. It was recently estimated that more people die annually from invasive fungal diseases than from tuberculosis (our unpublished data), and the prognosis for patients with invasive fungal infections has remained nearly unchanged in the last two decades. This fact, in combination with the central role played by a deficient host defense in the pathogenesis of fungal infections, has led to the concept that adjunctive immunotherapeutic approaches should be developed to improve the outcome of the disease. To achieve this goal, we need to understand the underlying mechanisms of protective antifungal immunity, and excitingly, this is one area where there have been significant recent advances. Here we introduce the major fungal pathogens that are associated with human disease and highlight some of the recent advances in our understanding of antifungal immunity.

The Pathogens

Only a few of the million or so known fungal species are truly pathogenic and capable of causing life-threatening infections in healthy individuals. The incidence of these diseases, such as histoplasmosis or coccidioidomycosis, is relatively low. In contrast, the majority of lethal invasive infections in immunocompromised hosts are opportunistic infections caused by normally commensal or saprophytic fungi, primarily species of *Candida*, *Cryptococcus*, *Aspergillus*, and *Pneumocystis*. In patients with AIDS, infections with *Pneumocystis jirovecii* and *Cryptococcus neoformans* (and the closely related *C. gatti*, sometimes also found to cause infection in healthy individuals) are the most frequent cause of fungal-related deaths, although non-life-threatening mucosal infections with *Candida* are also very common. Similarly, *Pneumocystis*, which causes life-threatening respiratory tract infections, and *Cryptococcus*, which preferentially targets the central nervous system, can also infect other immunosuppressed individuals, including transplant patients. Recent estimates suggest that there are 620,000 deaths per year in AIDS patients resulting from cryptococcal meningitis (Park et al., 2009), making *Cryptococcus* the most lethal fungal pathogen of man.

Noninvasive infections of the oral and genital mucosa are the most common form of infection with *Candida spp.*, but these microorganisms are also responsible for substantial numbers of invasive systemic and bloodstream infections in hospital settings, particularly in severely immunocompromised patients and patients undergoing invasive clinical procedures. In fact, *Candida* species, particularly *C. albicans*, are now the fourth most common cause of nosocomial bloodstream infections and the second leading cause of infectious-related death in extremely premature infants (Benjamin et al., 2010; Pfaller and Diekema, 2007). Species of *Aspergillus*, primarily *A. fumigatus* and *A. flavus*, cause significant numbers of invasive fungal infections in immunocompromised individuals and are one of the most feared fungal diseases, as they are very difficult to treat and have the highest rates of mortality. Individuals at risk include solid organ transplant recipients, neutropenic patients on strongly immunosuppressive therapies, or patients with defects in neutrophil function such as chronic granulomatous disease (CGD). *Aspergillus* also causes chronic pulmonary aspergillosis, a destructive disease complicating other pulmonary illnesses such as COPD, and allergic diseases, such as allergic bronchopulmonary aspergillosis. Several other species of fungi can also cause life-threatening infections, including *Histoplasma*, *Blastomyces*, *Rhizopus*,

Penicillium, and *Paracoccidioides*, but the incidence of each of these infections is less than those of the four species discussed above (our unpublished data).

Antifungal Immunity

Fungi have contributed significantly to our understanding of mammalian immunology for well over 100 years (Brown, 2010), yet major advances in our understanding of the mechanisms underlying protective antifungal immunity have occurred only relatively recently. Keeping with the topic of the reviews in this issue in this issue of *Cell Host & Microbe*, in this commentary we focus on pattern recognition receptors (PRRs), dendritic cells (DCs), Th17 immunity, and antibody responses, as these areas have provided substantial new insights and offer the potential for the development of novel immunotherapeutic approaches. We also aim to provide a snapshot of the central innate and adaptive components of protective antifungal immunity and to place in context the three associated reviews.

Innate Immunity: PRRs and DCs

Innate and adaptive immunity to fungal infections is critically dependent on phagocytic cells, and loss of these cells leaves individuals extremely vulnerable to infection. Neutropenia, for example, predisposes patients to life-threatening infections with several invasive fungal species (discussed above), while defects in neutrophil mobilization (due to defects in IL-17 function, for example) result in an increased predisposition to fungal infections at the mucosa. Phagocytes possess several oxidative and nonoxidative effector mechanisms, which are used synergistically to kill fungi, and defects in these mechanisms are also significant risk factors. Patients with inherited defects in their phagocyte NADPH oxidase, for example, develop CGD as they are unable to induce a respiratory burst and are predisposed to several life-threatening infections, including invasive aspergillosis (specially with *A. nidulans*). Importantly, the antifungal activities of phagocytes are strongly influenced by the state of cellular activation, which can be both substantially enhanced (by IFN-γ, for example) or suppressed (by IL-10 or steroids, for example) by soluble mediators.

Many of the antimicrobial functions of phagocytes are triggered following pathogen recognition by PRRs, and the discovery of these receptors, particularly the Toll-like receptors (TLR), was a significant advance. Intriguingly, although being originally discovered in relation to antifungal immunity in fruit flies, the exact role of individual TLRs in antifungal immunity in mammals is less clear. Indeed, there is contradictory evidence for the role of these PRRs for many fungal infections in experimental mouse models, and although polymorphisms in TLRs have been linked to susceptibility in humans, patients lacking essential downstream signaling components, such as MyD88, do not show obvious defects in antifungal immunity (von Bernuth et al., 2008). However, the TLRs collaborate with other PRRs, and while perhaps not essential in otherwise immunocompetent individuals, they do play critical roles in modulating the inflammatory and adaptive responses to fungal pathogens in the context of immunosuppression (Netea et al., 2008).

In contrast to the TLR, there is compelling evidence that other classes of PRR, particularly the C-type lectins (CLR) and NOD-like receptors (NLRs), play fundamental roles in protective antifungal immunity. CLRs such as DC-SIGN, the macrophage mannose receptor, Dectin-1, Dectin-2, and Mincle are involved in fungal tethering and uptake by phagocytes, induction of antifungal effector mechanisms, and the production of soluble mediators, including inflammatory lipids, cytokines, and chemokines. Importantly, like the TLRs, these receptors are capable of directing and modulating the development of adaptive immunity. Studies of the underlying mechanisms utilized by these receptors have also revealed novel signaling components, including the Syk/CARD9 pathway that is utilized by Dectin-1, Dectin-2, and Mincle. Importantly, polymorphisms or mutations in the CLRs or their intracellular signaling components have been linked to susceptibility to fungal infections in both mouse models and in humans (Netea and van der Meer, 2011). Of the NLRs, both the NLRP3 and NLRC4 have been found to play an essential role in antifungal immunity. These NLRs are components of inflammasomes, cytoplasmic multimeric proteolytic protein complexes that are involved in the processing and activation of IL-1β and IL-18. How these inflammasomes are actually induced by fungi is still unclear. More recently a noncanonical inflammasome involving caspase-8 has also been implicated, but here activation was shown to be mediated by Dectin-1 (Gringhuis et al., 2012). The roles and function of all these PRRs are discussed further in the associated reviews by Hernández-Santos and Gaffen (Hernández-Santos and Gaffen, 2012) and by Roy and Klein (Roy and Klein, 2012) in this issue.

Equally important to the discovery of PRRs was the finding that phagocytes, particularly DCs, mediate the induction and modulation of adaptive immunity. The unique ability of DCs to present antigen to naive T cells and drive the development of adaptive immunity has prompted substantial interest in identifying and characterizing the function of DC subsets. In mice (and humans), several types of DC have been identified, including plasmacytoid DCs, resident DCs, and migratory DCs, the latter consisting of several subsets that are found in different tissues, such as the skin, lung, and intestine. Importantly, these DC subsets have different functions, offering the possibility that directed targeting to these individual subsets can induce specific vaccine responses. In the accompanying review by Roy and Klein (Roy and Klein, 2012), the role and importance of the various DC subsets in antifungal immunity are explored as well as the recent advances in the targeting of these cells for the development antifungal vaccines. However, despite this progress, there are still no vaccines clinically available for any fungal pathogen.

Adaptive Immunity: Th17 and Antibody Responses

While the innate effector functions of phagocytes, particularly those of neutrophils and macrophages, are sufficient for protection against some infections, defense against most fungal pathogens also requires adaptive immune responses. As already mentioned, adaptive immunity is induced and modulated following interactions of microbes with phagocyte PRRs and the subsequent cytokine and chemokine profiles that are produced. In fact, interactions of fungi with particular PRRs have been directly linked to the induction of specific types of adaptive responses, including Th1 (TLR4, for example) and Th2 (TLR2, for example) immunity (Netea et al., 2008; Wüthrich et al., 2012). More recently, fungal recognition by CLRs and NLRs

has been shown to induce Th17 immunity (see below). Understanding the role of PRRs in driving these responses has had significant implications for our understanding of the mechanisms underlying the induction of protective antifungal adaptive immunity and the possibility of modulating immunity for vaccine design, topics that are discussed further in the associated reviews by Hernández-Santos and Gaffen (Hernández-Santos and Gaffen, 2012) and by Roy and Klein (Roy and Klein, 2012) in this issue.

Historically, inflammatory cytokines and Th1 responses were considered to provide protection against fungal infections, while immunosuppressive cytokines and Th2 responses were thought to contribute to susceptibility. This concept was supported by both human patients and animal models in which deficiencies in Th1 or inflammatory cytokines (such as IFN-γ or TNF, for example), or upregulation of Th2 or immunosuppressive cytokines (IL-4 and IL-10, for example) led to enhanced susceptibility to infections with various fungal pathogens (Romani, 2011). However, this distinction was not always clear-cut; for example, nonprotective cytokines, such as IL-10, are required to limit inflammatory pathology, in part by promoting the development of Tregs cells, whereas some level of IL-4 is needed to induce protective immunity. Furthermore, deficiencies in Th1 responses did not always correlate to susceptibility, especially for mucocutaneous fungal infections.

This latter paradox was solved recently following the identification of Th17 adaptive immunity, responses which were originally linked to autoimmunity. Interestingly, Th17 responses (characterized by the production of IL-17 and several other cytokines, including IL-22 and IL-23) appear to be primarily responsible for protection against fungal infections at the mucosa. The mechanisms for this protection are still being elucidated, but are thought to involve neutrophil recruitment and antimicrobial peptide production at the site of infection. These responses may also be partially required for the control of systemic infections caused by some (but not all) fungal pathogens, including *Candida*, *Aspergillus*, and *Cryptococcus* (Wüthrich et al., 2012). While there is contradictory evidence from certain mouse models, which suggest that inflammation induced during Th17 responses can contribute to pathology as well as susceptibility, defects in Th17 immunity have been directly linked to susceptibility to mucocutaneous fungal infections in man. The most common infection observed in these patients is chronic mucocutaneous candidiasis (CMC), and several other diseases previously associated with CMC, such as hyperimmunogolublin E syndrome and autoimmune polyendocrine syndrome 1, have now been linked to alterations in Th17 respones. A detailed account of Th17 responses in immunity to *Candida albicans* is presented in the review by Hernández-Santos and Gaffen (Hernández-Santos and Gaffen, 2012) in this issue of *Cell Host & Microbe*.

While it is clear that cell-mediated immunity is essential for resistance to fungal infections, humoral immunity has long been considered to have a secondary role. Indeed, studies looking at the passive transfer of immune sera or using B cell-deficient mice, for example, have failed to reliably demonstrate the importance of antibodies in antifungal immunity. However, the development and characterization of monoclonal antibodies (mAbs) has provided clear evidence that antibodies can mediate both protection and susceptibility (Casadevall and Pirofski, 2012b). Such opposing functions presumably counteract each other during the polyclonal responses initiated during fungal infections, explaining the earlier findings. Excitingly, however, protective mAbs could be used for the development of novel therapeutics, and protective antibody responses generated following the targeting of specific fungal antigens offers the potential for vaccine development. Indeed, antibodies to common antigens, such as β-glucans, could provide cross-species protection. The latest developments in this area are covered in detail in the review by Casadevall and Pirofski (Casadevall and Pirofski, 2012a) in this issue of *Cell Host & Microbe*.

Conclusions

The last few years have seen substantial advances in our understanding of antifungal immunity. Although a previously "neglected" area, these developments have promoted renewed interest in fungal infections and a rapid expansion of the field. Despite the important discoveries and successes of the last years, many of which were not discussed here, considerable challenges still remain. Developing vaccines that will be effective in immunocompromised patients is just one example. Furthermore, most attention has been focused on understanding the immunopathological mechanisms of the major invasive fungal pathogens that occur in immunocompromised patients in the developed societies, yet we still know little about the terrible endemic mycoses that affect thousands of individuals in the developing world or the common mucocutaneous infections that affect millions worldwide. No doubt exciting developments in these areas will be forthcoming in the future.

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REFERENCES

Benjamin, D.K., Jr., Stoll, B.J., Gantz, M.G., Walsh, M.C., Sánchez, P.J., Das, A., Shankaran, S., Higgins, R.D., Auten, K.J., Miller, N.A., et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. (2010). Pediatrics *126*, e865–e873.

Brown, G.D. (2010). Cell Host Microbe *7*, 9–11.

Casadevall, A., and Pirofski, L.-A. (2012a). Cell Host Microbe *11*, this issue, 447–456.

Casadevall, A., and Pirofski, L.A. (2012b). Nat. Immunol. *13*, 21–28.

Gringhuis, S.I., Kaptein, T.M., Wevers, B.A., Theelen, B., van der Vlist, M., Boekhout, T., and Geijtenbeek, T.B. (2012). Nat. Immunol. *13*, 246–254.

Hernández-Santos, N., and Gaffen, S.L. (2012). Cell Host Microbe *11*, this issue, 425–435.

Netea, M.G., and van der Meer, J.W. (2011). N. Engl. J. Med. *364*, 60–70.

Netea, M.G., Brown, G.D., Kullberg, B.J., and Gow, N.A. (2008). Nat. Rev. Microbiol. *6*, 67–78.

Park, B.J., Wannemuehler, K.A., Marston, B.J., Govender, N., Pappas, P.G., and Chiller, T.M. (2009). AIDS *23*, 525–530.

Pfaller, M.A., and Diekema, D.J. (2007). Clin. Microbiol. Rev. *20*, 133–163.

Romani, L. (2011). Nat. Rev. Immunol. *11*, 275–288.

Roy, R.M., and Klein, B.S. (2012). Cell Host Microbe *11*, this issue, 436–446.

von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C.L., Chrabieh, M., Mustapha, I.B., Ghandil, P., Camcioglu, Y., et al. (2008). Science *321*, 691–696.

Wüthrich, M., Deepe, G.S., Jr., and Klein, B. (2012). Annu. Rev. Immunol. *30*, 115–148.